Supplementary Note. Comparison against mit.edu genome-wide tracks and mit.edu web-based design tool.

To benchmark GuideScan we compared our database to those generated by other laboratories with published gRNA design tools. In **Figure 1b** and **Supplementary Figure 1a**, we used the genome-wide database generated by the Zhang laboratory at MIT (and publically released as UCSC tracks) as the basis for these comparisons. We refer to this database in our manuscript as mit.edu UCSC tracks. This resource was chosen for comparison because it is currently the only publically available genome-wide database of gRNAs. However, as stated by the authors on their website, the definition of an off-target used in the construction of this database was not completely predictive of Cas9 specificity. Consequently, the authors generated a new and improved design tool (which we refer to as the mit.edu web interface) that implements new and more stringent models to define off-targeting and to attribute specificity scores to gRNAs[11]. Thus, it is against this new tool that what we compare GuideScan's performance in terms of specificity in **Figure 2**, **Supplementary Figure 1b**, and **Supplementary Table 1**. As shown in **Figure 2a,** it appears that this new approach provides a higher density of gRNAs than what is provided by the UCSC tracks. However, because (like other web-based tools against which we compared GuideScan) the mit.edu web tool does not provide access to its underlying database, we were unable to use it for genome-wide comparisons, nor can experimenters use it to design high-throughput gRNA libraries.